

# Osteoarthritis and Cartilage



## PCB126 induces apoptosis of chondrocytes via ROS-dependent pathways

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### SUMMARY

**Objective:** Chondrocyte apoptosis represents an important component in the osteoarthritis (OA) pathogenesis. This study sought to investigate the potential of polychlorinated biphenyl (PCB)126, the most potent and ubiquitous environmental pollutant of PCB congeners, on chondrocyte apoptosis and its mechanism of action.

**Methods:** Rabbit articular chondrocytes cultured from tibial and femoral in cartilage were exposed to PCB126. Productions of reactive oxygen species (ROS) and nitric oxide (NO) and nuclear factor- $\kappa$ B (NF- $\kappa$ B) binding activity were measured. After 24 h exposure to PCB126, the apoptotic cell death was detected by caspase-3 activity, enzyme-linked immunosorbent assay (ELISA) using antibodies against DNA and histone, and terminal deoxynucleotidyl transferase (TdT)-mediated dUTP-biotin nick end-labeling (TUNEL) staining.

**Results:** PCB126 generated ROS, which was blocked by the antioxidants (*N*-acetylcysteine and trolox), or the aryl hydrocarbon receptor (AhR) inhibitor,  $\alpha$ -naphthoflavone ( $\alpha$ -NF). PCB126 exposure also increased NO production and NF- $\kappa$ B binding activity in the chondrocytes, which were blocked by the iNOS inhibitor, *N*-monomethyl-L-arginine (L-NMMA). All apoptosis detection techniques used in this study revealed an increase of apoptotic effects by PCB126 exposure, which was blocked by inhibitors of ROS or iNOS. This is the first report to demonstrate the potential of a PCB congener to induce chondrocytes apoptosis, which could be an initial process in cartilage degradation.

**Conclusions:** PCB may be an initiator of chondrocyte apoptosis, which is closely linked to degradation of cartilage in OA pathogenesis. This study may contribute to identifying the possible causes of arthritis in our environment.

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### Introduction

Polychlorinated biphenyls (PCBs) are a large class of aromatic chlorinated hydrocarbons comprising 209 congeners that differ in the number and position of chlorine atoms. PCBs are widely distributed environmental contaminants that have been found at many different levels in the food chain. These chemicals are persistent and bioaccumulative in the body and cause a wide range of tissue- and species-specific toxic effects such as carcinogenicity, teratogenicity, immune suppression, and endocrine disruption.<sup>1</sup> While many human health risks from exposure to PCBs and related compounds are well documented,<sup>2</sup> a possible link with joint diseases is reported only in a limited number of human studies. The “Yusho” patients in Japan who were accidentally exposed to high

levels of PCBs and furans after ingestion of contaminated rice oil commonly showed swelling of the joints and arthralgia.<sup>3,4</sup> The “Yucheng” cohort from a PCB exposure incident in Taiwan has arthritis four times higher than the unexposed subjects.<sup>5</sup> Recently, a strong positive association between background levels of PCB exposure and arthritis in women was reported.<sup>6</sup> These studies strongly suggest that PCBs may play a role in the pathogenic processes of arthritis.

Destruction of the cartilage matrix by a pathological imbalance of normal chondrocyte function is a key element in the progression of arthritis, in particular, osteoarthritis (OA).<sup>7</sup> The chondrocyte is the only cell type in articular cartilage and plays an essential role in determining the integrity of the cartilage. Thus, chondrocyte cell death is responsible for cartilage damage, which is the most prominent feature of arthritis.<sup>8</sup> Chondrocyte apoptosis is more frequently observed among advanced OA cases than in normal subjects.<sup>9</sup> Clinical specimens from OA cartilages showed reduced cellularity or increased apoptotic chondrocyte death.<sup>10</sup> Because apoptotic cell death is a critical event in the pathogenesis of joint diseases,<sup>11,12</sup> identification of apoptosis inducers is considered a key

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element in understanding the causes of arthritis. However, it remains unclear what initiates the apoptotic process of chondrocytes at the early stage of arthritis and what is the underlying mechanism.

Because PCBs have been associated with arthritis<sup>3,5,6</sup> and functions of chondrocytes have clinical importance in the pathogenesis of arthritis,<sup>12</sup> we hypothesized that PCB126, the most potent congener of coplanar PCBs, induces apoptotic processes of chondrocytes which may lead to the initiation of cartilage damage. This study examined effects of PCB126 on chondrocyte apoptosis and its related mechanistic pathways in order to understand a potential link between environmental pollutant exposure and joint diseases such as arthritis.

## Materials and methods

### *Chondrocyte culture, treatment and study design*

Rabbit articular chondrocytes were cultured from tibial and femoral in cartilage as described previously.<sup>13,14</sup> Briefly, articular cartilages were removed from 2-week-old New Zealand White rabbits (Samtako Biokorea Co., Korea). Dissected cartilage slices were dissociated enzymatically for 4 h in 0.2% collagenase type II (Sigma, St Louis, MO) in Dulbecco's modified Eagle's medium (DMEM) (Gibco BRL, Grand Island, NY), and single cells were obtained by collecting the supernatant after centrifugation at 300 g for 5 min. Quality of chondrocytes was determined by examining the accumulation of sulfated glycosaminoglycan or the expression of type II collagens (data not shown). The chondrocytes were exposed to various concentrations of PCB126 (>99.7% purity; AccuStandard, New Haven, CT) as further described below. One animal was used to perform each independent experiment. Within each experiment, individual measurements were performed in triplicate to test the reliability of analysis technique. Each experimental value represents mean of triplicate measurements at each concentration. Mean of three independent experimental values ( $n = 3$ ) was presented.

### *MTT assay*

The tetrazolium salt 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT; Sigma, St Louis, MO) assay was performed to determine the cell viability of chondrocytes after 12 h, 24 h or 36 h exposure of PCB126, as described in Hirata.<sup>15</sup>

### *Measurement of reactive oxygen species (ROS)*

Formation of intracellular ROS was measured using a fluorescent probe, 2',7'-dichlorofluorescein diacetate (DCFH-DA) as described by Mariussen *et al.*,<sup>16</sup> with minor modifications.<sup>14</sup> Chondrocytes were seeded at a density of  $2.8 \times 10^5$  cells/mL in 35 mm dishes and maintained in the growth medium for 6 days at 37°C. Cells were treated with DMSO (0.1%), 0.01, 0.1, 1  $\mu$ M PCB126 for 15 min at 37°C in 5% CO<sub>2</sub> incubator. For the pretreatment studies, cells were exposed with 10  $\mu$ M  $\alpha$ -naphthoflavone ( $\alpha$ -NF), 100  $\mu$ M trolox, or 10 mM *N*-acetylcysteine (NAC) 1 h prior to the exposure of PCB126, respectively. Concentrations of additives used in the study have been previously described.<sup>16</sup> Fluorescence was recorded (excitation wavelength 485 nm, emission wavelength 530 nm) at 37°C for 1 h.

### *Measurement of nitric oxide (NO) production*

NO production was measured by estimating the stable NO metabolite, nitrite, in conditioned medium using a spectrophotometric method based on the Griess reaction.<sup>17</sup>

Chondrocytes cultured on 96-well plates ( $2 \times 10^4$  cells/well) for 6 days were treated with DMSO (0.1%), 0.01, 0.1, 1  $\mu$ M PCB126 for 24 h. For the inhibition study, cells were pretreated with 0.5 mM *N*-monomethyl-L-arginine (*L*-NMMA), 1 h prior to PCB exposure.

### *Nuclear factor- $\kappa$ B (NF- $\kappa$ B) activity*

The activation of the NF- $\kappa$ B p65 was determined using a TransAM NF- $\kappa$ B p65 Chemi kit according to the manufacturer's instructions (Active Motif, Carlsbad, CA). Cells in serum-free media were treated with PCB126, in the presence or absence of 0.5 mM *L*-NMMA for 24 h. The nuclear pellet was collected and resuspended in complete lysis buffer (TransAM kit) and the protein concentration was determined using a Bradford assay (Sigma–Aldrich, St Louis, MO). The concentration of the activated transcription factor was detected using an enzyme-linked immunosorbent assay (ELISA)-based method. The nuclear extract was incubated for 1 h on a plate coated with an oligonucleotide that corresponded to a transcription factor consensus site. The wells were washed and incubated with an antibody against the indicated transcription factor subunit. An anti-IgG HRP conjugate was added and then the developing solution was added. Then, the chemiluminescent reaction was measured using a MicroLumat Plus LB 96V luminometer (Berthold Detection System, Oak Ridge, TN).

### *Measurement of caspase-3 activity*

Caspase-3 activity was determined with the ApoAlert caspase colorimetric assay kit (Clontech Laboratories, Palo Alto, CA), as described previously.<sup>18</sup> Briefly, chondrocytes were incubated for 24 h with PCB126 in the presence or absence of 10 mM NAC or 0.5 mM *L*-NMMA. Protease activity was quantified by a microplate reader (BioRad, Hercules, CA) at 405 nm.

### *Analysis of apoptosis by ELISA*

Fragmented nucleosomal DNA was quantified by an ELISA kit (Cell Death Detection ELISA Plus; Roche, Mannheim, Germany) as described in the manufacturer's manual. Briefly,  $4 \times 10^5$  cells were plated in 35 mm culture dishes in the presence of 10% FBS with antibiotics (streptomycin and penicillin). After 6 days, the cells were exposed to DMSO (0.1%), 0.01, 0.1, 1  $\mu$ M PCB126 for 24 h in the presence or absence of 10 mM NAC or 0.5 mM *L*-NMMA.

### *Terminal deoxynucleotidyl transferase (TdT)-mediated dUTP-biotin nick end-labeling (TUNEL) assay*

DNA fragmentation was detected with a TUNEL assay kit (fluorescein *in situ* cell death detection kit; Roche, Mannheim, Germany) according to the manufacturer's protocol. In brief, chondrocytes grown on coverslips were exposed to DMSO (0.1%), 0.01, 0.1, 1  $\mu$ M PCB126 for 24 h in the presence or absence of 10 mM NAC or 0.5 mM *L*-NMMA. TUNEL-positive cells were counted under a Zeiss Axiophot fluorescence microscope (Zeiss, Oberkochen, Germany). The number of TUNEL-positive cells was then divided by the total number of cells in the field.

### *Statistical analysis*

GraphPad Prism software (ver. 4.0) was used to derive mean and 95% confidence interval (CI) (lower and upper limits) of three independent experiments ( $n = 3$ ) performed in triplicate. Mann–Whitney test was performed to determine a statistical

difference between control and treated groups. A *P*-value of less than 0.05 was considered significant and presented in the text.

## Results

### Cytotoxicity

The MTT assay was used to determine cytotoxicity of PCB126 on articular chondrocytes at different time points. 12 h exposure of PCB126 did not affect cell viability of chondrocytes except 10  $\mu$ M, which showed a 70% cell survival of the untreated cells ( $P = 0.045$ ). There were no significant increases of floating cells or debris detached from the surface of culture dishes at concentrations up to 1  $\mu$ M. 24 h exposure showed 22% and 45% reduction of cell viability at 1  $\mu$ M ( $P = 0.042$ ) and 10  $\mu$ M ( $P = 0.032$ ), respectively. 36 h exposure significantly dampened cell viability at all concentrations except 0.01  $\mu$ M ( $P = 0.046$  at 0.1  $\mu$ M, 0.032 at 1  $\mu$ M and 0.008 at 10  $\mu$ M). Since cell death can be initiated as apoptosis,<sup>19</sup> the exposure time of 24 h and concentrations up to 1  $\mu$ M, which did not show a drastic reduction of cell viability, were selected in this study for optimal detection of apoptotic events and NO production (Fig. 1).

### ROS generation

Significant increases of ROS generation were observed following 15 min exposure to PCB126 [% of control; 177 (95% CI 171–183;  $P = 0.025$ ) at 0.01  $\mu$ M, 227 (95% CI 221–233;  $P = 0.008$ ) at 0.1  $\mu$ M and 242 (95% CI 238–247;  $P = 0.008$ ) at 1  $\mu$ M] and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) [% of control; 123 (95% CI 117–129;  $P = 0.045$ ) at 0.001  $\mu$ M, 136 (95% CI 130–142;  $P = 0.043$ ) at 0.01  $\mu$ M and 170 (95% CI 161–179;  $P = 0.009$ ) at 0.1  $\mu$ M] [Fig. 2(A)]. The increase by PCB126 was blocked by the pretreatment of 10 mM NAC ( $P = 0.008$ ), 100  $\mu$ M trolox ( $P = 0.006$ ) or 10  $\mu$ M  $\alpha$ -NF ( $P = 0.005$ ) [Fig. 2(B)]. These results suggest that PCB126 is an effective stimulant of aryl hydrocarbon receptor (AhR)-dependent ROS generation in rabbit chondrocytes.

### NO generation

NO is a key element in modulating apoptosis of chondrocyte. Dioxin, which shares a mechanism of action with PCB126, is known

to increase NO production in chondrocytes.<sup>14</sup> Thus, this study looked into the potential of NO production with PCB126 exposure, which may mediate apoptosis of chondrocyte. Chondrocytes exposed to PCB126 increased NO production [% of control: 115 (95% CI 110–120) at 0.01  $\mu$ M, 153 (95% CI 144–162;  $P = 0.034$ ) at 0.1  $\mu$ M and 208 (95% CI 196–220;  $P = 0.004$ ) at 1  $\mu$ M] that was effectively blocked by 0.5 mM L-NMMA, iNOS inhibitor ( $P = 0.041$ ) (Fig. 3).

### NF- $\kappa$ B activity

NF- $\kappa$ B binding activity of PCB126 was evaluated to understand the inflammatory responses in chondrocytes. The relative amount of active transcription factor was measured using the TransAM Active Motif NF- $\kappa$ B kit. The amount of activated NF- $\kappa$ B family members was significantly increased by PCB126 [% of control; 98 (95% CI 95–101) at 0.01  $\mu$ M, 208 (95% CI 200–216;  $P = 0.013$ ) at 0.1  $\mu$ M and 274 (95% CI 265–283;  $P = 0.002$ ) at 1  $\mu$ M]. This increase was blocked by L-NMMA ( $P = 0.042$  at 0.1  $\mu$ M and 0.009 at 1  $\mu$ M) (Fig. 4).

### Apoptotic cell death

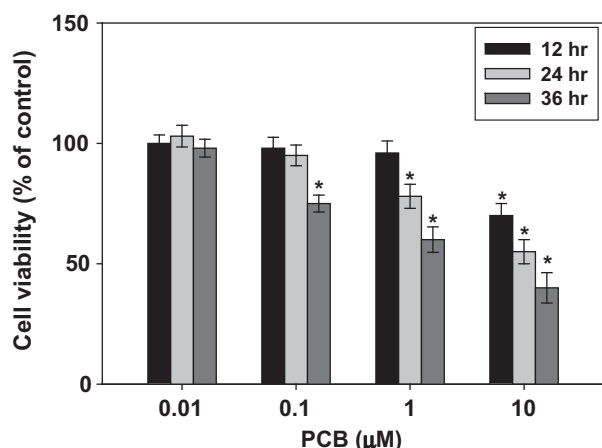
Since any single method is not reliable enough to characterize apoptotic cells properly,<sup>11,20</sup> three different technical approaches were performed in this study. After 24 h exposure to PCB126, the apoptotic cell death was detected by caspase-3 activity, ELISA using antibodies against DNA and histone, and TUNEL staining. Activity of caspase-3, an executioner caspase of apoptosis, was increased following exposure to PCB126 [% of control: 125 (95% CI 119–131) at 0.01  $\mu$ M, 188 (95% CI 180–196;  $P = 0.034$ ) at 0.1  $\mu$ M and 250 (95% CI 241–259;  $P = 0.008$ ) at 1  $\mu$ M] [Fig. 5(A)]. ELISA analysis showed increases [% of control: 98 (95% CI 94–102) at 0.01  $\mu$ M, 115 (95% CI 111–119;  $P = 0.048$ ) at 0.1  $\mu$ M and 151 (95% CI 146–156;  $P = 0.018$ ) at 1  $\mu$ M] of histone-associated DNA fragments [Fig. 5(B)]. Fluorescence microscopy using TUNEL staining revealed an increased detection of DNA strand breaks with PCB126 exposure (Fig. 6). The number of TUNEL staining positive cells was significantly increased following exposure to PCB126 [% of control (number of positive cells with DMSO; 100%): 190 (95% CI 179–201) at 0.01  $\mu$ M, 290 (95% CI 275–305;  $P = 0.042$ ) at 0.1  $\mu$ M and 410 (95% CI 383–435;  $P = 0.008$ ) at 1  $\mu$ M]. Increase of these apoptosis parameters was dampened with prior treatment of 10 mM NAC or 0.5 mM L-NMMA (Figs. 5 and 6). While NAC blocked increase of both caspase-3 activity and DNA fragmentation at 0.1  $\mu$ M ( $P = 0.045$ ) and 1  $\mu$ M ( $P = 0.038$ ), L-NMMA inhibited these apoptotic parameters at 1  $\mu$ M only ( $P = 0.036$ ) (Fig. 5). The results suggest that ROS may be more effectively involved in induction process of chondrocyte apoptosis than NO.

## Discussion

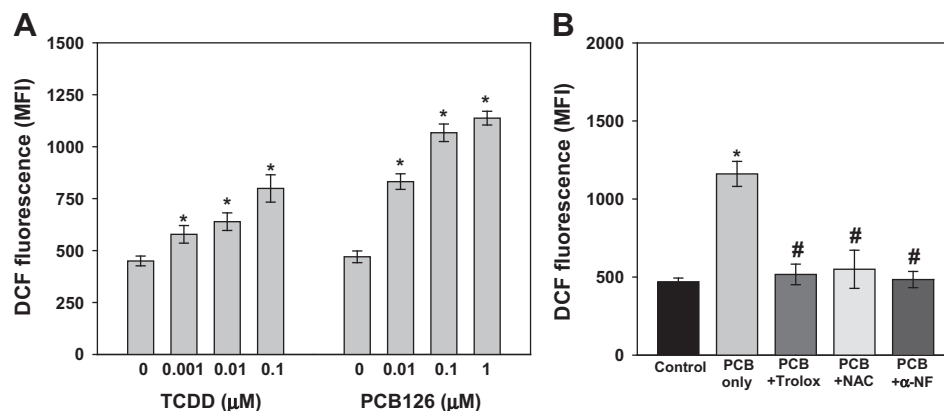
Human studies suggest that PCBs and related compounds are associated with the occurrence of joint disease. PCBs accumulate in the human body and interact with cells of the skeletal system.<sup>21</sup> However, no attempt has been made to investigate effects of PCBs in chondrocytes. This is a first report demonstrating apoptotic effects of PCB in chondrocytes including some evidence for possible mechanism of action.

PCB congeners are classified into coplanar or non-coplanar PCBs by their structural moiety. While coplanar PCBs mediate their toxic effects by binding to the AhR, non-coplanar PCBs exhibit AhR-independent effects.

The structural moiety of PCBs possibly related to arthritis in human studies is coplanar type, which has a dioxin-like mechanism of action. In this study, PCB126 was used to determine apoptotic



**Fig. 1.** Cell viability of chondrocyte following PCB126 exposure. Chondrocytes plated on 96-well plate were treated with different concentrations of PCB126 for 12 h, 24 h and 36 h. Cell viabilities were measured with MTT assay as described in the text. All values are relative to the control cells (the viability of chondrocyte with DMSO; 100%) and represent mean and 95% CI (error bar) from three independent experiments. Statistical difference from DMSO control (\*) was set to  $P < 0.05$ .



**Fig. 2.** Effects of PCB126 and TCDD on ROS generation. Cells were treated with 0.1% DMSO, TCDD (0.001, 0.01, 0.1 μM) or PCB126 (0.01, 0.1, 1 μM) for 15 min, respectively (A). Cells were treated with PCB126 only or in the presence of antioxidants (100 μM trolox; 10 mM NAC) or AhR blocker (10 μM α-NF) (B). Relative fluorescence was measured as described in method section. All unit values represent mean and 95% CI (error bar) of three independent experiments. Statistical difference from DMSO control (\*) and PCB126 only-treated cells (#), respectively, was set to  $P < 0.05$ .

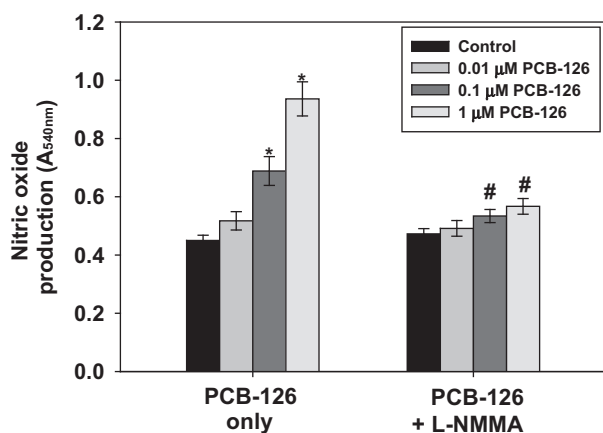
effects in chondrocytes because it is the most potent congener of coplanar PCBs.

Our previous study demonstrated that chondrocytes effectively respond to sensitive biomarkers of dioxin exposure and contain a battery of dioxin-responsive genes, suggesting that a primary chondrocyte model can be effectively used to elucidate a mechanistic pathway of dioxin-induced toxicity in cartilage tissues.<sup>14</sup> Because PCB126 has a mechanism similar to dioxins, the primary chondrocyte model may be a suitable tool to characterize similar cellular effects following exposure to PCB126.

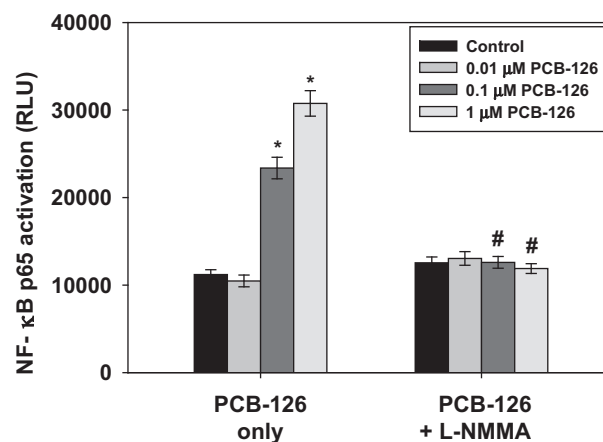
Different cell viabilities over exposure time points in this study suggest that exposure duration is an important factor in PCB-induced cell death of chondrocytes in culture (Fig. 1). While decreases of cell viability after 24 h exposure were not reflected in a similar fashion with increases of apoptotic parameters, 36 h exposure revealed a pattern of cell viability similar to apoptotic responses observed after 24 h exposure (Fig. 1). Because cell death can be initiated as apoptosis in a continuous spectrum of cell death partially overlapping both apoptosis and necrosis,<sup>19,22</sup> different patterns of outcomes between apoptosis and cell viability assays after 24 h may be due to the fact that higher number of cells is in an early apoptotic stage than in necrotic stage at this time point.

ROS mediates damage of the extracellular matrix by induction of chondrocyte apoptosis in cartilage.<sup>23</sup> This study demonstrated that PCB126 is an effective inducer of ROS generation in chondrocytes (Fig. 2), indicating that PCBs may play a role in the process of cartilage damage via a ROS-mediated pathway. It is worthy to note that ROS generation by PCB126 was higher than TCDD at the molar concentration basis, while toxic equivalent factor (TEF) of PCB126 is 10 times lower than that of TCDD. Thus, it is speculated that ROS-mediated cellular impact by PCBs may be higher than dioxin in chondrocytes and ROS generation may be an important element for PCB mechanism of action in the process of cartilage damage. Toxicity of dioxins, in general, is higher than that of PCBs. But, only PCBs have been suggested as a possible cause of joint disease in human studies. Higher potency of ROS generation in our results may explain, in part, why PCBs could be more associated with progression of cartilage damage than dioxin and support epidemiological findings which link arthritis or joint pains with PCB exposure.

NO plays a key role in various signaling pathways in physiological processes. Excess NO, in general, is known to contribute to a detrimental outcome of joint diseases.<sup>24</sup> Overproduction of NO was detected in synovial tissues and articular cartilage in arthritic

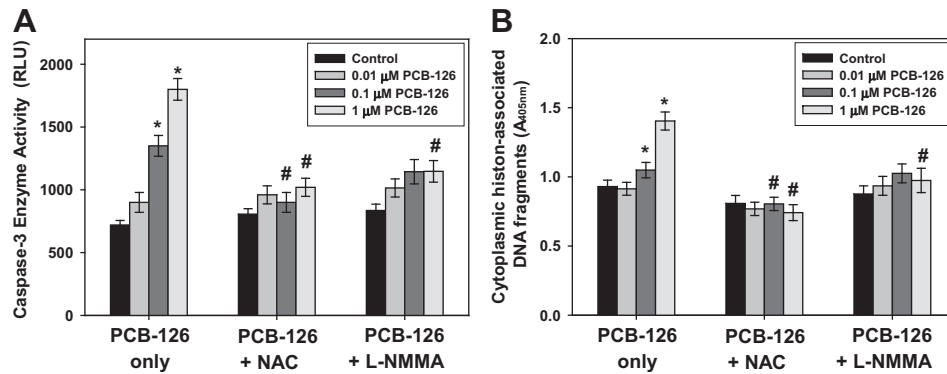


**Fig. 3.** Effects of PCB126 on NO generation. Cells were treated with 0.01, 0.1 and 1 μM PCB126 in the presence or absence of 0.5 mM L-NMMA. NO production was measured as described in method section. Unit values are mean and 95% CI (error bar) from three independent experiments. Statistical difference from DMSO control (\*) and PCB126 only-treated cells (#), respectively, was set to  $P < 0.05$ .



**Fig. 4.** Effects of PCB126 on NF-κB binding activity. Cells were treated with 0.01, 0.1 and 1 μM PCB126 in the presence or absence of 0.5 mM L-NMMA. NF-κB binding activity was measured as described in method section. Unit values are mean and 95% CI (error bar) from three independent experiments. Statistical difference from DMSO control (\*) and PCB126 only-treated cells (#), respectively, was set to  $P < 0.05$ .





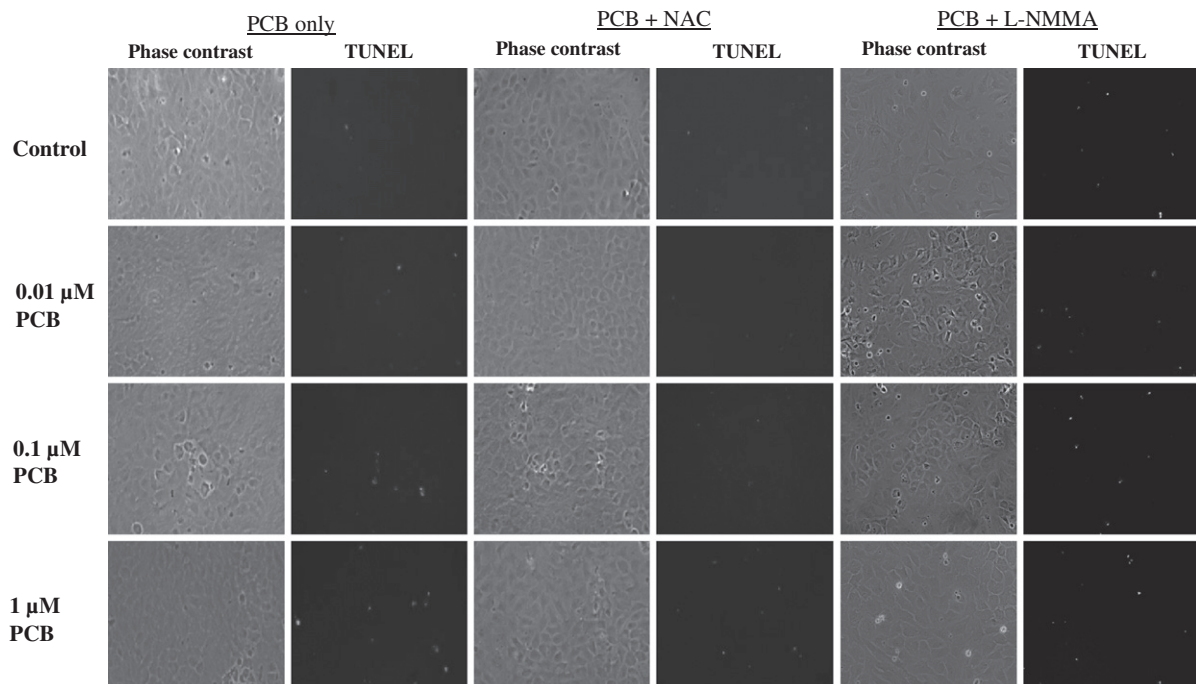
**Fig. 5.** Effects of PCB126 on apoptosis induction. Apoptosis of chondrocyte was quantified by caspase-3 activity (A) and ELISA (B), as described in method section. Cells were exposed to 0.01, 0.1, 1 μM PCB126 for 24 h in the presence or absence of 10 mM NAC or 0.5 mM L-NMMA. All the values represent mean and 95% CI (error bar) of three independent experiments. Statistical difference from DMSO control (\*) and PCB126 only-treated cells (#), respectively, was set to  $P < 0.05$ .

patients.<sup>25</sup> In this study, L-NMMA blocked an increase of NO production and apoptotic parameters induced by PCB126, suggesting that NO may play an important role in PCB-mediated apoptosis (Figs. 3 and 5). Further studies are required in the future to determine the type of reactive nitrogen derivatives involved in apoptosis.

NF-κB is an inducible factor that regulates various physiological processes including the inflammatory responses. It plays a crucial role in arthritis, mediating important chondrocyte inflammatory responses that ultimately lead to cartilage degradation.<sup>26,27</sup> It is reported that elevated NF-κB signaling in chondrocytes contributes to cartilage degradation in OA<sup>28</sup> and a two-fold increase of NF-κB binding activity was observed in OA chondrocytes, as compared to normal ones.<sup>27</sup> The 2.8-fold increase of NF-κB binding activity observed in this study (Fig. 4) suggests that PCB126 may play a role in cartilage degradation via the NF-κB activation pathway. NO is a potent modulator of NF-κB activation. However, its role is cell-type specific and depends on experimental conditions.<sup>27</sup> Thus, it is

important to examine the relationship between NO production and NF-κB binding activity to understand the possible mechanism of PCB effects in chondrocytes. PCB126 exposure revealed an increase of NO production and iNOS inhibitor blocked its NF-κB binding activity. The results indicate that NO production by PCB may mediate NF-κB signaling pathway, which plays a central role in pro-inflammatory stress-related responses of chondrocytes.<sup>28</sup> In future studies, it would be important to clarify whether PCB-mediated NF-κB activation is associated with chondrocyte apoptosis.

Chondrocytes in culture are reported to undergo apoptosis in response to various stimuli including serum deprivation, NO donor, cytokines, PGE<sub>2</sub>, etc.<sup>7</sup> However, induction of chondrocyte apoptosis by environmental pollutants such as PCBs remains unknown. We used a panel of the different technical approaches to obtain the optimal detection of PCB-induced chondrocyte apoptosis. Although a certain difference of sensitivity between methods exists, all three different methods clearly demonstrated that PCB126 is a potent inducer of chondrocyte apoptosis (Figs. 5 and 6). Apoptosis was



**Fig. 6.** Fluorescence microscopic image of TUNEL staining. Representative images from three independent experiments are presented. Magnification 400×.

increased in a dose-dependent manner following the PCB exposure, as measured by caspase-3 activity and nucleosomal DNA fragmentation assay. These apoptotic measures were further confirmed by analyzing microscopic images of TUNEL staining (Fig. 6). Inhibitory effects of apoptosis by NAC were more apparent than those of L-NMMA (Figs. 5 and 6), implicating that ROS may be more effective mediator than NO in PCB-induced apoptosis. Our results are consistent with the previous reports that both ROS and NO play important roles in mediation of chondrocyte apoptosis.<sup>23,29,30</sup> The apoptosis-inducing effects observed in *in vitro* systems may not be pathologically implicated as inducers of joint diseases such as arthritis. Therefore, caution must be taken in interpretation of *in vitro* data regarding their pathophysiological relevance.<sup>7,16</sup> *In vitro* responses are variable depending on the species, condition of the donor and the culture conditions. Despite these limitations, it is noteworthy that PCB126, the most potent congener of this class, was identified as an apoptosis inducer of articular chondrocytes.

Among environmental pollutants detected in humans, none has ever been suggested as a possible cause of arthritis in human epidemiological studies except PCBs. Therefore, this study may contribute to establishing the scientific data basis for assessing risk of environmental pollutants for arthritis and add biological plausibility to the recent human study. Although the significance of our findings should be validated in subsequent animal studies, this study provides initial evidence that persistent environmental agents such as coplanar PCBs may play a role in the etiology of cartilage disease through induction of chondrocyte apoptosis. Our results may shed a new light in studying the role of environmental pollutants in the etiology of arthritis.

## Contributions

All authors made substantial contributions to the concept and design of this study, acquisition of experimental data, drafting the manuscript and the final approval of the contents to be submitted.

## Conflict of interest

All authors declared no competing financial interest regarding this manuscript and agreed that the work is ready for the submission to the journal and accept responsibility for the manuscript's contents.

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